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EFFECTS OF THE POTENTIAL ANTIDEPRESSANT OPC-14523 A COMBINED SIGMA AND 5-HT$_{1A}$ LIGAND: MODULATION OF NEURONAL ACTIVITY IN THE DORSAL RAPHE NUCLEUS

by

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Running Title: Effects of OPC-14523 on Dorsal Raphe Neurons

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Abbreviations: 4-IBP- 4-(N-benzylpiperidin-4-yl)-4-iodobenzamide, 5-HT-serotonin, 8-OH-DPAT- 8-hydroxy-2-(di-n-propylamino)tetralin, DRN- dorsal raphe nucleus, DTG- 1,3-di-(2-tolyl)guanidine, GABA- -aminobutyric acid, JO-1784-(+)-N-cyclopropylmethyl-N-methyl-1,4-diphenyl-1-1-ethyl-but-3-en-1-ylamine hydrochloride, MAOI-monoamine oxidase inhibitor, NE-100- N,N-dipropyl-2-(4-methoxy-3-(2-phenylethoxy)phenyl)-thylamine, NMDA-N-methyl-D-aspartate, OPC-14523- 1-[3-[4-(3-chlorophenyl)-1-piperazinyl]propyl]-5-methoxy-3,4-dihydro-2-quinolinone monomethanesulfonate, p-CPA- chlorophenylalanine, SA-4503- 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl) piperazine dihydrochloride, SSRI- selective serotonin reuptake inhibitor, (+)SKF-10,047- (+)-N-allyl-normetazocine, WAY-100635-(N-[2-(4-[2-methoxyphenyl]-1-piperazinyl)ethyl]-N-2-pyridinylcyclohexanecarboxamide.

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ABSTRACT

OPC-14523 (OPC) is a novel compound with high affinity for sigma and 5-HT_{1A} receptors as well as for the 5-HT transporter. OPC has previously been shown to produce antidepressant-like effects in animal models of depression. This project set out to determine OPC's effect on serotonergic neurotransmission and to shed light on its mechanism(s) of action. In an electrophysiological model of *in vivo* extracellular recordings in anesthetized rats, a 2-day treatment (1 mg/kg/day), with OPC induced a significant increase in dorsal raphe nucleus (DRN) putative 5-HT neurons' firing activity. This increase was blocked by the co-administration of NE-100, a selective $sigma_1$ antagonist (10 mg/kg/day). Furthermore, after 2-day treatments with OPC, the 5-HT_{1A} autoreceptor response was altered, as demonstrated by the dramatically reduced response to an increase of endogenous 5-HT induced by the acute administration of paroxetine (500 μg/kg i.v.). However, the 5-HT_{1A} agonist 8-OH-DPAT (4 μg/kg i.v.) maintained its ability to decrease 5-HT firing activity, an effect which was reversible by the subsequent administration of the 5-HT_{1A} antagonist WAY 100635 (100 μg/kg i.v.). As 8-OH-DPAT has been shown to act preferentially through postsynaptic 5-HT_{1A} receptors, our data suggests that this effect of OPC is mediated primarily by the 5-HT_{1A} autoreceptor. The decreased response of the 5-HT_{1A} autoreceptor to paroxetine was not blocked by the co-administration of NE-100, indicating that $sigma_1$ receptors are not involved in this effect. Thus, both sigma and 5-HT_{1A} receptors play a role in the "antidepressant-like" effects produced by OPC, which is in keeping with previously published behavioural data. In addition, the current series of experiments suggest that OPC might have potential as an antidepressant with a rapid onset of action, as compared to SSRI treatments, which initially suppress the firing activity of putative 5-HT neurons and require at least 2-3 weeks in order to restore the firing activity to baseline neuronal firing activity through a desensitization of the 5-HT_{1A} autoreceptor**.**

1. INTRODUCTION

An enormous amount of evidence suggests the involvement of the serotonin system in the pathophysiology of depression (Reviewed by Delgado, 2000). Electrophysiological data demonstrates that representatives from all classes of antidepressants, after long-term treatments and through various mechanisms, increase 5-HT neurotransmission (Chaput et al., 1991; Blier and de Montigny, 1994). For example, acute treatments with monoamine oxidase inhibitors (MAOI's) and selective serotonin reuptake inhibitors (SSRI's) lead to decreased firing activity of 5-HT neurons in the dorsal raphe nucleus (DRN), but as treatment continues, the 5-HT neurons regain normal firing activity due to desensitization of the 5-HT_{1A} somatodendritic autoreceptor. This desensitization has been proposed as the adaptive change that explains the delayed enhancement of 5-HT-mediated neurotransmission, which is consistent with the clinical onset of action of SSRI's (Chaput et al., 1986; Blier et al., 1988; Blier and de Montigny, 1994).

The existence of sigma receptors was initially reported by Martin et al., (1976). The existence of at least two receptors, denoted sigma$_1$ and sigma$_2$ is now accepted (Quirion et al., 1987, 1992). Sigma ligands have been implicated in the pathophysiology of depression or have been proposed as potential antidepressants. Sigma ligands such as SA-4503, (+)-pentazocine, DTG and JO-1784 have been shown to produce antidepressant-like effects in behavioural models of depression such as the Forced Swimming Test and Tail Suspension Test and in clinical trials (Matsuno et al., 1996; Tottori et al., 1997; Kinsora et al., 1998; Ukai et al., 1998; Akunne et al., 2001; Pande et al., 1998).

We previously demonstrated that the sigma ligands 4-IBP and (+)-pentazocine produce an increase in the basal firing activity of 5-HT neurons of the DRN after both short-term (2 days) and long-term (21 days) treatments (Bermack and Debonnel, 2001). The effects of (+)-pentazocine were blocked by the co-administration of the selective sigma$_1$ antagonist NE-100, while those of 4-IBP were blocked by the co-administration of the non selective sigma antagonist haloperidol, but not NE-100 (Bermack and Debonnel, 2001).

OPC is a novel compound with high affinities for sigma and 5-HT_{1A} receptors and with 5-HT reuptake inhibitory activities (Tottori et al., 2001). Similar to other sigma ligands, OPC yielded

antidepressant-like effects in animal models of depression (Tottori et al., 2001). Interestingly, the immobility time was reduced in the forced swimming test after a single dose of OPC and the daily administration for 7 days enhanced this effect (Tottori et al., 2001).

Thus, the purpose of this study was to assess the effect of acute and short-term treatments with OPC on DRN neurotransmission using an electrophysiological model of extracellular recordings of putative 5-HT neurons from the DRN. Furthermore, we assessed the effect of the treatments with OPC on the response of the 5-HT_{1A} somatodendritic autoreceptors since OPC has high affinity for 5-HT_{1A} receptors, and this could have relevance to its potential antidepressant properties.

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2. MATERIAL AND METHODS

2.1. Animals

Experiments were performed in male Sprague-Dawley rats (Charles River, St. Constant, Québec) weighing 250-300g. Rats were housed under standard laboratory conditions including 12-12hr light-dark cycle and free access to food and water**.**

2.2. Acute Treatments

For the acute treatments, once a putative 5-HT neuron was identified and recorded for approximately 1 minute, saline or OPC were administered intravenously via the tail vein during electrophysiological recording. Five rats were tested for each dose studied, with one injection administered per rat.

2.3. Short-term Treatments

Animals were anesthetized with halothane for the subcutaneous implantation of osmotic minipumps (Durect Corporation, Cupertino, CA, USA) placed in the back of the animal. The minipumps delivered a dose of 1 mg/kg/day of OPC-14523 dissolved in 5% ethanol and distilled water. A separate series of rats were implanted with 2 pumps simultaneously; one containing OPC and the other containing NE-100 (10 mg/kg/day). The duration of all treatments were 2 days. The control groups were treated with minipumps filled with 5% ethanol and distilled water. Electrophysiological experiments were performed with the minipump(s) on board.

2.4. Electrophysiological experiments

Experiments were performed on rats anesthetized with chloral hydrate (400 mg/kg, i.p.). Supplemental doses of chloral hydrate (100 mg/kg i.p.) were administered as needed to prevent any nociceptive reaction to pinching of the hind paw. The rat's body temperature was maintained at approximately 37 °C by a thermistor-controlled heating pad.

A 2 mm-diameter section of bone centered on 1 mm anterior to Lambda was removed from the skull. A glass micropipette, tip diameter 1-3 μm, filled with 1 M NaCl (impedance 2-4 MΩ) was lowered vertically, and 200 m-spaced tracks covering the DRN were performed. Spontaneously active neurons of the DRN were encountered starting from the ventral border of the Sylvius aqueduct and down to 1 mm below. Putative 5-HT neurons, which constitute the vast majority of spontaneously

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active DRN neurons, were identified according to classical physiological parameters characterized by the simultaneous occurrence of triphasic, positive-going first, action potential waveform; spike duration >2 ms; slow (0.2-3.5 Hz) and clock-like discharge pattern (Aghajanian, 1978; Aghajanian et al., 1978). Following the experiments each rat was sacrificed with anesthetic overdose.

2.5. Data Collection

For acute treatments, the mean firing rate before and after the injection of OPC was compared to assess any acute effects of OPC on the firing activity. For each short-term treatment group, the mean DRN 5-HT firing rate was determined by averaging the firing activity of all the neurons per group. For each treatment group (OPC, OPC and NE-100), the total number of neurons contributing to the average was greater than 50 from a minimum of 4 rats. To assess the effect of paroxetine or 8-OH-DPAT, the percentage of inhibition was calculated and the average for each of each drug dose was determined. Statistical analysis was performed with the software SigmaStat for Windows Version 4.0 (Jandel Corporation). One-way ANOVA was used with alpha=0.05, followed by a post-hoc analysis using Tukey's Method of comparison versus controls. Results (F) of statistical analysis are expressed in terms of degrees of freedom between groups (df) and number of groups compared (p). $P < 0.05$ was considered statistically significant for all tests.

2.6. Drugs

8-OH-DPAT (8-hydroxy-2-(di-n-propylamino) tetralin) and paroxetine HCl were purchased from Sigma Aldrich Canada Limited (Oakville, ON, Canada). OPC was provided by Otsuka Pharmaceutical Co. Ltd (Tokushima, Japan), NE-100 was a gift from Taisho Pharmaceutical Co. Ltd, (Tokyo, Japan).

3. RESULTS

The doses used were chosen based on results obtained in behavioural tests with OPC (Tottori et al. 2001) and on previous results with other sigma ligands in our electrophysiological paradigm (Bermack and Debonnel 2001). In control animals, putative 5-HT neurons were encountered starting at a depth of 4616 μm, with an average firing rate of 1.03 Hz.

3.1. Acute Treatments

To assess any potential acute effect of OPC on neuronal activity in the DRN, we compared the effect of an acute intravenous dose of 20 μg/kg of OPC to saline. This dose had no effect on the firing activity of putative DRN 5-HT neurons. Similarly, when administered at a dose of 50 μg/kg, OPC did not modify the firing activity (data not shown).

3.2. Average Firing Rate

We then assessed whether OPC would have any effects if administered for a short-term treatment. Following two-day treatments with OPC (at a dose of 1 mg/kg/day), the mean basal firing activity of putative 5-HT neurons was increased by 50% (as compared to controls 1.45±0.11 Hz n=51 vs. 1.02±0.07 Hz n=56 [$F(2,3)=5.80$ $P<0.05$ Tukey Test $q=4.81$]) (Figure 1). The co-administration during the two days of OPC and the $sigma_1$ antagonist NE -100 at a dose of 10 mg/kg/day prevented the increased firing activity observed with OPC alone such that the firing rate was not significantly different versus controls (1.21 ± 0.10 Hz n=54, vs. 1.02 ± 0.07 Hz n=56 [$F(2,3)=5.80$, n.s.])(Figure 1).

3.3. Modulation of 5-HT Neuronal Activity by 5-HT_{1A} Autoreceptors

Since 5-HT_{1A} autoreceptors constitute a key element in the control of the firing activity of 5-HT neurons and since OPC has a high affinity for 5-HT_{1A} receptors we also assessed the function of the 5-HT_{1A} autoreceptor in rats treated for 2 days with OPC. To this end, we assessed the effect of the acute administration of the SSRI paroxetine (500 μg/kg, i.v.). Figure 2 depicts representative tracings of putative DRN serotonergic neurons recorded during administrations of paroxetine, a 5-HT_{1A} agonist (8-OH-DPAT) and a 5-HT_{1A} antagonist (WAY 100635). In control animals, paroxetine induced a near complete suppression of the firing activity of DRN neurons (Figure 2A). This effect

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was reversed by the subsequent administration of the 5-HT_{1A} antagonist WAY-100635 (Figure 2A). In animals treated with OPC-14523 for two days, the effect of paroxetine was drastically reduced (compared to controls 9.98 ± 4.47% n=5 vs. 99.4 ± 0.40% n=5 [$F(3,4)=123.18$ $P<0.05$ Tukey's test $q=23.51$]) (Figures 2C and 3). Increasing the dose of paroxetine by 3 folds (up to 1500 μg/kg i.v.), only increased the degree of suppression to about 16% compared to 99% with 500 μg/kg in the control animals (1500 μg dose compared to controls 16.40 ± 2.58% n=5 vs. 99.4 ± 0.40% n=5 [$F(3,4)=123.18$ $P<0.05$ Tukey's test $q=21.83$]) (Figure 3). In animals treated for two days with a combination of OPC-14523 and NE-100, the effect of paroxetine was identical to that observed in rats treated with OPC alone (500 μg dose compared to controls 3.94 ± 0.79% n=5 vs. 99.4 ± 0.40% n=5 [$F(3,4)=829.72$ $P<0.05$ Tukey's test $q=62.64$]) (Figures 2D and 3).

In animals treated for 2 days with a dose of 1 mg/kg/day of OPC and following 3 doses of paroxetine, a small dose of 8-OH-DPAT (4 μg/kg, i.v.) produced a drastic reduction of the firing activity of DRN neurons, very similar to what was observed in control animals (compared to controls 72.2 ± 4.31% n=5 vs. 68.2 ± 10.13% n=5 [$F(2,3)=1.14$ n.s.]) (Figures 2C and 4). The effect of 8-OH-DPAT was reversed by the subsequent administration of WAY-100635 (100 μg/kg, i.v.) (Figures 2C and 4).

Finally, in animals treated with the combination of OPC and NE-100 for 2 days, 8-OH-DPAT induced the same degree of inhibition of the firing activity of DRN neurons as in control rats or in animals treated with OPC alone (compared to controls 58.2 ± 4.04% n=5 vs. 68.2±10.13% n=5 [$F(2,3)=1.14$ n.s.]) (Figures 2D and 4).

4. DISCUSSION

OPC, in addition to being a sigma$_1$ ligand (IC_{50}=47-56nM), has affinity for the 5-HT_{1A} receptor (IC_{50}=2.3nM) and for the 5-HT transporter (IC_{50}=27 nM) (Tottori et al., 2001). The current experiments found that acute treatments with OPC (20 and 50 μg/kg) did not produce any change in the firing activity of 5-HT neurons. However, following 2-day treatments with OPC (1 mg/kg/day) the firing activity of putative 5-HT dorsal raphe neurons was increased by 50% (Figure 1). Interestingly, this effect of OPC on the basal firing activity DRN neurons was reversed by NE-100 (Figure 1).

The increase in firing rate observed after 2 days of treatment with OPC is in agreement with previous data from our laboratory showing that 2-day treatments with the sigma ligands 4-IBP and (+)-pentazocine to induce approximately a 35% increase in the firing activity (Bermack and Debonnel, 2001). The fact that this increase is prevented by the co-administration of NE-100, suggests an effect mediated via a subtype of sigma$_1$ receptors, presumably the same one mediating the effects of (+)-pentazocine observed previously, which induces an equal increase in the firing activity of dorsal raphe neurons following a 2-day treatment, an effect which was also suppressed by NE-100 (Bermack and Debonnel, 2001).

In addition to inducing an increase in the firing activity, OPC treatments also prevented the suppressant effects of paroxetine (500 μg/kg, i.v.) as shown by a dramatic decrease in the response to this SSRI (Figure 3). Interestingly, following 2-day treatments with OPC, the effect of the acute intravenous administration of the 5-HT_{1A} agonist 8-OH-DPAT (i.e the reduction of the firing activity of 5-HT neurons) was still present (Figure 4). In contrast with the effects of a two-day treatment with OPC on the basal firing activity, its effects on paroxetine action was not prevented by a co-treatment with NE-100, suggesting that this effect is not mediated by the sigma$_1$ receptors but is rather due to OPC's affinity for 5-HT_{1A} receptors.

It could also be suggested that OPC's effects on paroxetine are due to a blockade of 5-HT reuptake by OPC. However, this appears unlikely since it would suggest a very potent blocking effect on the 5-HT transporter since 1 mg/kg/day would be as potent as 10-15 mg/kg/day of other SSRI's paroxetine, fluoxetine and citalopram. Whereas, in *ex vivo* experiments, OPC at doses of 100 and 300

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mg/kg p.o. were required to inhibit H^3-5-HT reuptake in rats versus doses of 10 or 30 mg/kg of fluoxetine. Furthermore, fluoxetine (10 or 30 mg/kg) blocked the pCPA-induced decrease in 5-HT content in the hippocampus or frontal cortex while OPC required 400 mg/kg to produce the same effect (Tottori et al., 2001). Finally, if OPC was acting mainly via its reuptake properties one would expect it to have a similar profile as other SSRI's when administered acutely, ie. to induce a decrease in firing activity of 5-HT neurons, however the acute administration of OPC (20 or 50 μg/kg) produced no change in the firing activity.

Thus, our data suggest that OPC's effects on the paroxetine response are due to its affinity for 5-HT_{1A} receptors. 5-HT acts upon several subtypes of receptors (Reviewed in Hoyer and Martin, 1996; Barnes and Sharp, 1999), with the 5-HT_{1A} receptors being particularly important in the regulation of 5-HT neurons' activity. Activation of these receptors triggers the opening of K^+ channels, which induces a hyperpolarization of the neuron and decreases its firing activity. The acute administration of SSRIs initially induces a moderate increase in the concentration of 5-HT in the vicinity of 5-HT cell bodies, thus triggering an activation of somatodendritic autoreceptors and a reduction of the firing activity of the 5-HT neurons, through a negative feedback mechanism (Gardier et al., 1996; Chaput et al., 1986; de Montigny et al., 1981; Aghajanian, 1978).

Numerous evidence has shown that the postsynaptic 5-HT_{1A} receptor, although very similar to the autoreceptor [since there is only one gene coding for the two types (Reviewed in Hoyer and Martin, 1996; Barnes and Sharp, 1999)] presents a different pharmacological profile. Some examples are; chronic SSRI and 5-HT_{1A} agonist treatments desensitize the 5-HT_{1A} somatodendritic autoreceptor in the DRN (Le Poul et al., 2000; Kreiss and Lucki, 1995; Blier et al., 1990), but do not change the responsiveness of postsynaptic 5-HT_{1A} receptors in the hippocampus (Le Poul et al., 2000; Chaput et al., 1986), and, agonist-induced internalization of 5-HT_{1A} receptors occured only presynaptically, but not postsynaptically (Riad et al., 2001). These differences could be related to the difference in the G-proteins linked to the receptors (Hensler, 2002).

Postsynaptic 5-HT_{1A} receptors also contribute to the control of the firing activity of 5-HT neurons through a long negative feedback loop (Pineyro and Blier, 1999). This system is not yet fully characterized but is initiated by the activation of medio-prefrontal-cortical postsynaptic 5-HT_{1A}

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receptors on glutamatergic neurons, leading to the activation of GABAergic interneurons in the DRN (Celada et al., 2001; Martin-Ruiz and Ugedo, 2001; Haddjeri et al., 2000; Tada et al.,1999; Hajos et al., 1999,1998, 2003; Ceci et al., 1994). It has been shown that the inhibitory effect of the systemic administration of the 5-HT_{1A}agonist 8-OH-DPAT is mediated preferentially via this long feedback loop, through an activation of postsynaptic 5-HT_{1A} receptors (Celada et al., 2001; Hajos et al., 1998,1999; Ceci et al., 1994).

OPC does not appear to affect the postsynaptic 5-HT_{1A} receptors since 2-day treatments with OPC did not modify the effects of 8-OH-DPAT, which as stated above exerts preferentially its inhibitory effects of DRN firing activity through activation of 5-HT_{1A} postsynaptic receptors through the feedback loop just described. However, further investigation of OPC's effects, studied at postsynaptic sites would further clarify the pharmacological profile of OPC at postsynaptic 5-HT_{1A} receptors. Therefore, the effects of OPC on paroxetine-induced inhibition of firing appear to be mediated via the 5-HT_{1A} autoreceptor. They could either be the result of a very rapid desensitization of the receptor with an agonist or of the blockade of the receptor by an antagonist.

5-HT_{1A} agonistic properties of OPC have been previously reported, based on the ability of OPC to induce a flat body posture (Tottori et al., 2001). However, this effect was observed at a dose of 30 mg/kg whereas, the ED_{50} for the same effects with 8-OH-DPAT was of 0.05 mg/kg, when the two compounds present about the same affinity for the 5-HT_{1A} receptor (Oshiro et al., 2000). Thus, the behavioural effect observed could be due to weak agonist effects at postsynaptic receptors or to a non-selective effect of OPC as such high doses could involve activation of other types of receptors for which OPC has lower affinity. Such an effect would be difficult to reconcile with a rapid desensitization. Moreover, if OPC was acting as an agonist on the 5-HT_{1A} autoreceptor one would expect a decrease of the firing activity of DRN neurons following its acute administration.

Conversely, an antagonist effect of OPC on 5-HT_{1A} autoreceptors could easily explain all the results observed in the present experiment. As, following the acute administration, as a 5-HT_{1A} antagonist it would have no effect on the spontaneous firing activity, moreover, through this antagonistic effect it would also prevent any inhibition of the firing due to an increase of endogenous

5-HT induced by the 5-HT transporter blocking properties of OPC. The same effect would be responsible for the decreased response to paroxetine following 2-day treatments.

Our results therefore suggest that besides its ability to block 5-HT uptake which was not assessed in the present series of experiments, OPC presents several aspects, each of them being by itself suggestive of a potential antidepressant effect. In conclusion, with the combination of all these aspects it could be expected that OPC, by inducing a rapid decreased response of the autoreceptor in addition to inducing an increase in the firing activity of DRN neurons, could represent a potent antidepressant with a rapid onset of action. Further studies into OPC's exact pharmacological effects on 5-HT_{1A} sites using microiontophoretic studies, which are now underway, will elucidate the mechanism underlying its observed effects on the autoreceptors. In addition, it remains to be established that OPC will keep a similar profile following a long-term treatment, which is what would be ultimately required with depressed patients.

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FIGURE LEGENDS

Figure 1

(A) Representative spontaneous firing rate histograms of dorsal raphe 5-HT neurons in (1) control rats, (2) rats treated for 2 days with OPC (1 mg/kg/day) and (3) rats treated for 2 days with OPC (1 mg/kg/day) and NE-100 (10 mg/kg/day). Each group of spikes represents a 90 second recording of the firing activity, thus, for example A1, represents recordings from 3 separate neurons encountered in one descent in the DRN. The number on top of each neuron represents the depth at which the neuron was found in μm. Time calibration applies to all traces.

(B) Mean firing activity of dorsal raphe 5-HT neurons in control rats expressed in Hz ± SEM (open column), in rats treated for 2 days with OPC (1 mg/kg/day) (grey column) and in rats treated for two days with a combination of OPC (1 mg/kg/day) and the $sigma_1$ antagonist NE-100 (10 mg/kg/day) (black column). In this and all subsequent figures the number in the box in each column represents the number of neurons contributing to the average.* $p < 0.05$.

Figure 2

Spontaneous firing rate histograms of dorsal raphe 5-HT neurons in (A) control rats, illustrating the suppression of the firing activity by the intravenous administration of the SSRI paroxetine and the reversal of this suppression following the subsequent administration of the $5\text{-}HT_{1A}$ antagonist WAY-100635. (B) control rats, illustrating suppression of firing activity by the intravenous administration of the $5\text{-}HT_{1A}$ agonist 8-OH-DPAT and reversal of this suppression following the subsequent administration of WAY-100635. (C) Illustrates the reduction of the effect of paroxetine in a rat treated for 2 days with OPC (1 mg/kg/day) and that the effect of 8-OH-DPAT is maintained and reversed by the subsequent administration of WAY-100635. In (D), a two-day combined treatment with OPC and the $sigma_1$ antagonist NE-100 induces the same effects. Time calibration applies to all traces.

Figure 3

Responsiveness expressed as the degree of suppression of the mean firing activity of dorsal raphe 5-HT neurons by the acute intravenous administration of 500 µg/kg of paroxetine in control rats (open columns), and of successive doses of 500 µg/kg of paroxetine in rats treated for 2 days with OPC-14523 (1 mg/kg/day) (light grey columns), or rats treated for 2 days with OPC-14523 (1 mg/kg/day) and the $sigma_1$ antagonist NE-100 (10 mg/kg/day) (dark grey columns). * $p < 0.05$. Note that the number of neurons tested per dose when not shown due to space is the same for all doses.

Figure 4

Responsiveness expressed as the degree of suppression of the mean firing activity of dorsal raphe 5-HT neurons by the acute intravenous administration of 4 µg/kg of 8-OH-DPAT in control rats (open columns), and in rats treated for 2 days with OPC (1 mg/kg/day) (grey column) or OPC (1 mg/kg/day) and the $sigma_1$ antagonist NE-100 (10 mg/kg/day) (black column).

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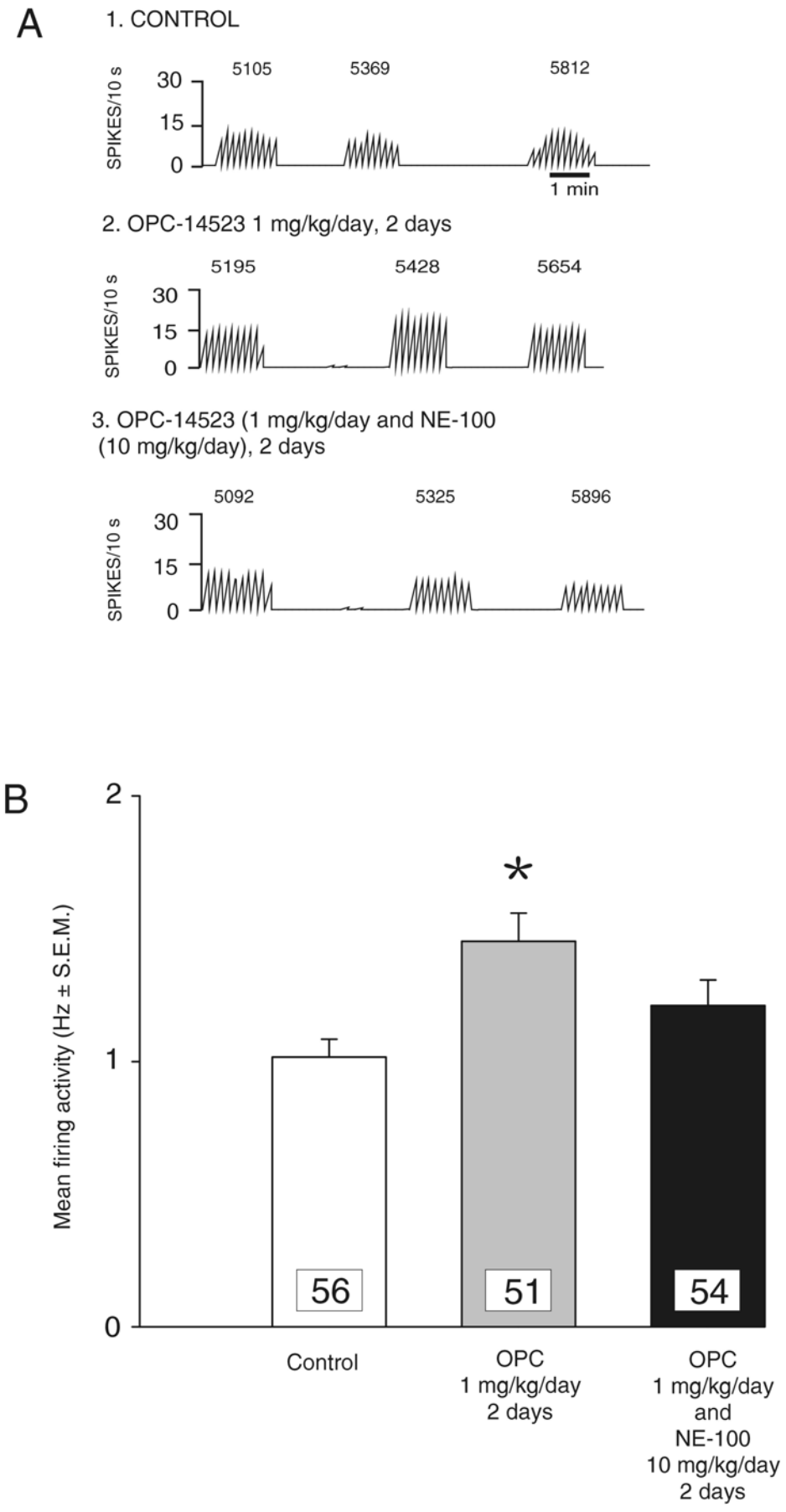

Figure 1

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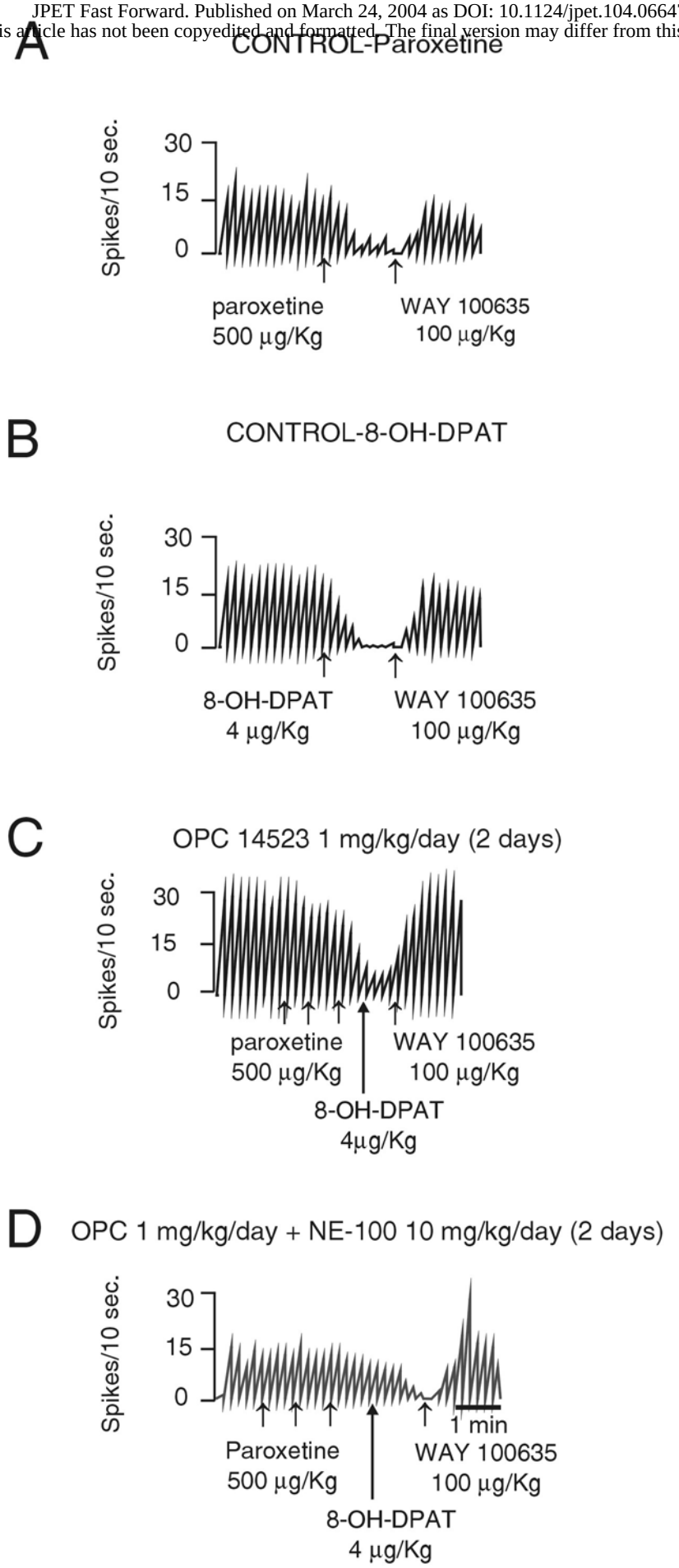

Figure 2

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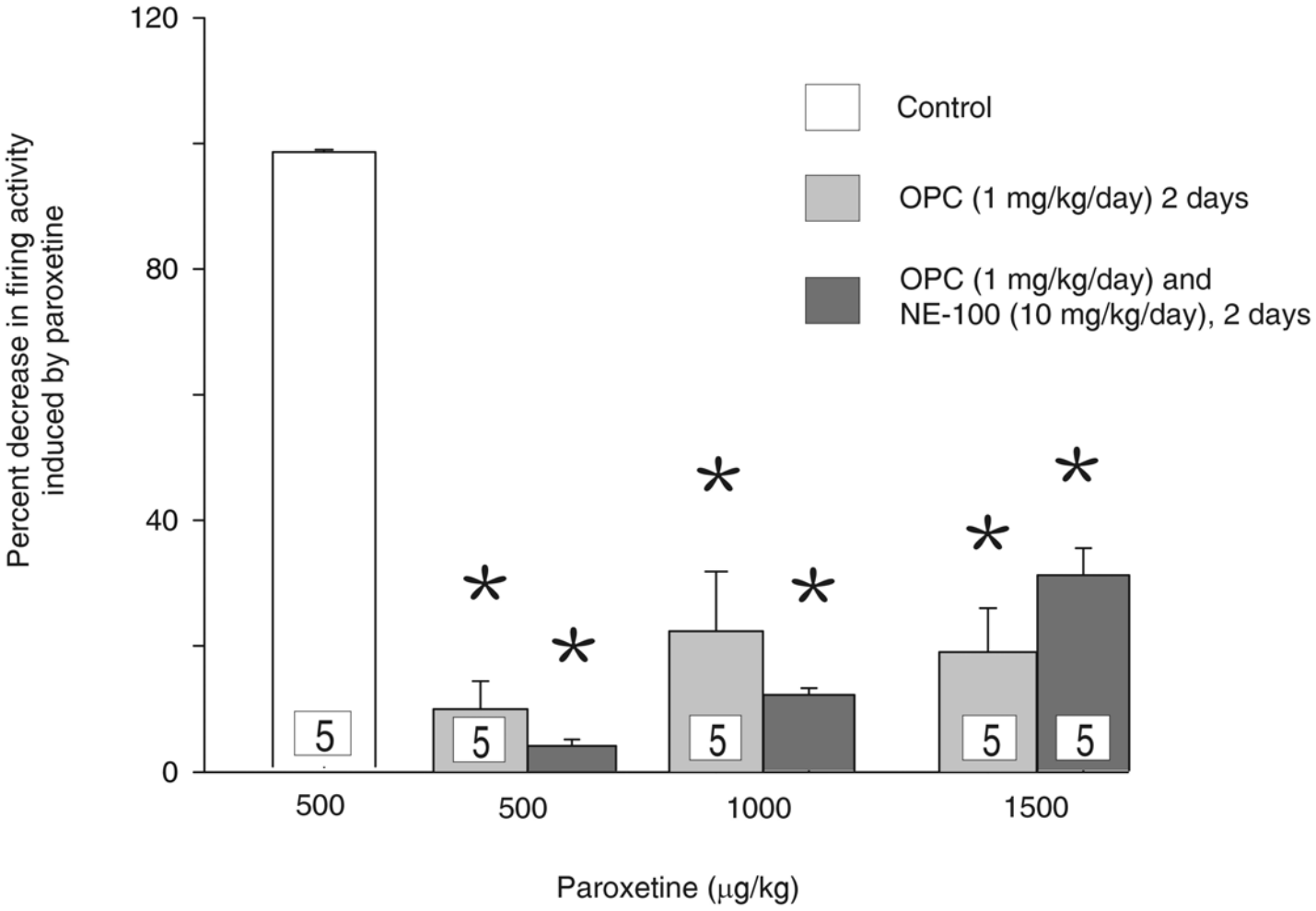

Figure 3

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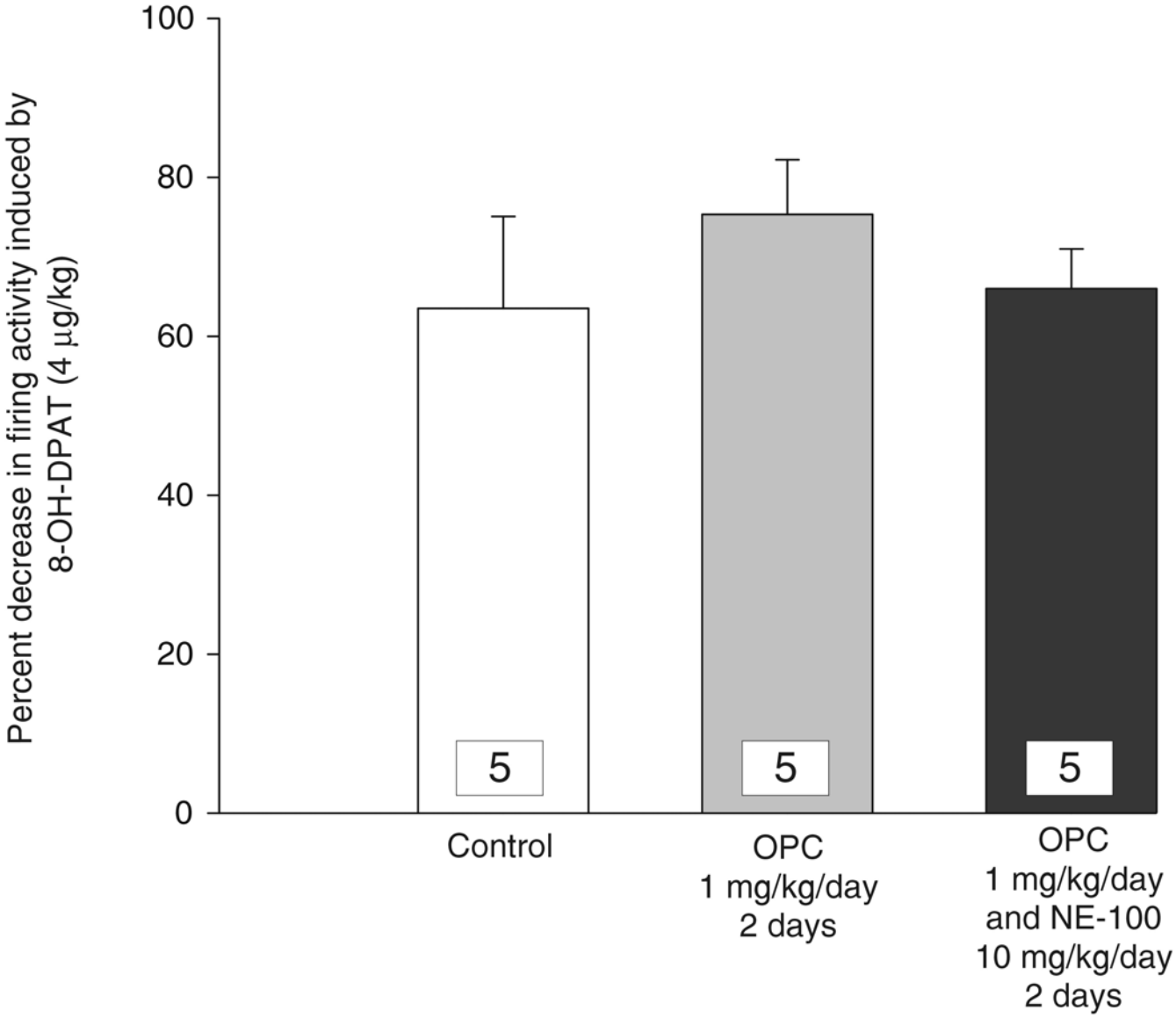

Figure 4